Quantification of the Cellular Components of Breast Duct Lavage Samples

Dear Dr. Bedrossian:

In 1985, Drs. Dupont and Page published a pivotal study suggesting a 5.3-fold increased breast cancer risk among women with biopsy-proven atypical proliferative breast disease, compared with women whose biopsies revealed nonproliferative breast lesions.[1] Among women with biopsy-proven hyperplasia without atypia, the relative risk was lower, that is, a 1.9-fold increase.[1] In a large prospective study of several thousand women in the San Francisco Bay area, Wrensch et al. reported that cytologically-diagnosed hyperplasia and atypical hyperplasia of cells derived from breast nipple aspirate fluid (NAF) were associated with an increased risk of breast cancer, and that the relative risk of breast cancer was higher in women with breasts that actually yielded NAF compared with those that did not.[2] These findings have been confirmed more recently with a similar group of patients.[3] Based on these studies, it was inferred that the breast ducts yielding NAF were actually the specific ducts, at risk of developing breast cancer. However, the actuality of this theory remains speculative, at best.[4–6]

Recently, there has been renewed interest in devising methods for sampling the resting breast duct epithelium. The goals of these efforts include breast cancer risk classification, early breast cancer detection, earlier recognition of within-breast cancer recurrence, biomarker development, and etiologic research. Techniques that access large surface areas of ductal epithelium also have the potential capacity to be used as conduits for novel drug delivery systems.

Breast duct lavage (BDL) is a relatively new technology, which has been devised to harvest breast duct cells. The major impetus behind this technique was the development of a microcatheter, which permitted irrigation of the cannulated duct, under local anesthesia. Using this microcatheter-based, saline lavage technique, exfoliated breast duct cells could be obtained to both assess biomarkers of risk assessment/neoplasia, and to evaluate cellular morphology. BDL, aimed at sampling breast ducts which yield NAF, has been shown to detect abnormal intraductal breast cells 3.2 times more often than nipple aspiration alone.[7] Given that histologic atypia represents a risk factor for the development of carcinoma, and the hypothesis that cytologic atypia has the same implications for breast cancer risk as histologic atypia, BDL may also represent a way to follow ductal epithelial atypia over time.[8]

BDL was originally promoted to the public as a "Pap Smear for the Breast,"[9] despite major differences between the two techniques. A properly-done Pap smear samples a representative portion of the entire transition zone of the cervix, while a BDL procedure can potentially, at best, sample only several of the proximal portions of the 15–20 ducts that terminate in the nipple.[10,11] As pointed out by Badve, there is a fundamental anatomical flaw with this sampling technique: breast ducts often exist in a collapsed state, since they do not have a strong wall.[12] This ductal collapse interferes with postlavage fluid retrieval, whereby only half of the infused fluid injected can be collected for analysis (e.g., 5 ml retrieved after 10 ml injected).[7,12]

As experience with this procedure has grown, it appears that BDL may have greater utility as a risk assessment tool rather than a diagnostic procedure, with eligibility determined by increased epidemiologic risk for breast cancer, as established by the Gail or Claus models.[13] In a recent editorial, Linder states that duct lavage should *not* be equated with breast FNA, that is, definitive action and surgery should not be based on the results of a duct lavage alone.[13] O'Shaughnessy has published management recommendations based on BDL results, which are outlined in Table I.[14]

The proposed diagnostic classification of BDL cytology results parallels that of breast FNA, that is, benign, atypia

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Table I. Management Recommendations for Ductal Lavage Results[14]

Cytologic interpretation	*Recommended management*
Benign	Repeat lavage in 1–3 yr.
Mildly atypical	Repeat lavage within 1 yr or consideration of prevention therapy.
Markedly atypical or malignant	• Additional studies to confirm the results, e.g., MRI, ductoscopy, or ductogram. • If a lesion is found a tissue biopsy may be performed. • Consideration of prevention therapy.

(mild or marked), malignant, and inadequate. "Mild atypia" corresponds to the spectrum associated with hyperplasia to atypical hyperplasia, and "marked atypia" corresponds to the spectrum of disease from atypical hyperplasia to high-grade ductal carcinoma in situ.[15] Specific morphologic features of these diagnostic categories have been suggested by Ljung,[15] although these definitions have not yet been subjected to rigorous validation.

In fact, serious questions loom regarding interobserver reproducibility of BDL cytology. In our experience, this is particularly problematic in the "benign" and "mild atypia" diagnostic categories. This was one of the several areas of study in a recent publication.[4] Statistically, the interobserver agreement was reported as "good;" however, there was complete diagnostic agreement among the three participating cytopathologists in only 9 of the 29 mastectomy-derived BDL samples they reviewed (31%), with partial agreement (2 of 3 agreeing) for only 17 of 29 samples (58%).[4]

The clinical utility of this technique has become more uncertain since its original promulgation. A recently-published study, in which BDL was performed on 38 breasts with proven cancer, yielded NAF in 29 cases; markedly atypical or malignant cells were reported in 5 of the 38 (13%) cases, and mildly to markedly atypical cells in 16 of the 38 (42%).[6] This range of diagnostic sensitivity is unacceptably low for use as a potential screening tool.[6,16] A previously-published study showed that BDL had a low sensitivity for the detection of carcinoma: the diagnosis of malignancy was not made in any BDL performed intraoperatively on 16 breasts with histologically-proven DCIS and an intact breast duct system.[4]

Thus, the ultimate **clinical** utility of BDL has yet to be determined, because of issues related to sample adequacy, imprecise cytologic interpretation, and uncertain prognostic implications associated with the diagnosis of "atypia." To meet the various **research** goals of BDL, large numbers of duct cells must be captured. Methods of counting constituent cells have not been standardized. In a previous report, median cell counts of 13,500 cells/duct with a range of 43–492,000 encouraged investigators to believe that this procedure will yield sufficient number of cells for translational research purposes.[7] We herein report what we believe to be an objective, accurate, reproducible method for evaluating the cellularity of BDL samples. A total of 37 archival cases was utilized for this review.

These 37 BDL samples were obtained as part of an ongoing, IRB-approved clinical research protocol (NCI Protocol 01-C-009) sponsored by the Clinical Genetics Branch of the National Cancer Institute. Informed consent was obtained from all study participants. BDL specimens were processed and evaluated as follows:

- A Thinprep® (TP) was prepared from the entirety of each BDL sample (~15 ml).
- Each slide was reviewed for epithelial cell clusters with ≥10 ductal cells.
- All such cell clusters were counted directly, with cell numbers within each cluster estimated in multiples of 10.
- These numbers were added to yield the total count of breast duct cells in large clusters.

Since there are three hundred thirty 20× fields on a TP slide (personal communication, Gary Gill, C.T., A.S.C. list serve, 6/18/02), single duct cells, cells in clusters <10, and histiocytes were counted as follows:

- Ten consecutive 20× fields were viewed down the middle of the TP slide.
- For each 20× field, the number of ductal cells and histiocytes were counted separately and summed.

For the final cell count and cellular proportions the following formula was utilized:

Epithelial cells in clusters	(a)
(+)33 (No. of epithelial cells in ten 20× fields)	(b)
(+)33 (No. of histiocytes in ten 20× fields)	(c)
Total no. of cells	a + b + c
Total no. of ductal cells	a + b

Thus, the total number of duct epithelial cells is calculated via the simple addition of a + b. The percentage of the sample that comprised of breast duct epithelial cells can then be calculated by dividing that number by the total number of cells (c). (Figs. C-1–C-3).

Of these 37 cases, 10 (27%) were deemed unsatisfactory for cell enumeration, and 2 were "satisfactory" (>10 ductal cells), but had too few cells for this counting method. Of the 25 samples with adequate cell counts, the breast duct epithelial cell count ranged from 73 to 40,000. *The mean number of epithelial cells per sample was 10,000*. The percentage of the sample that contained ductal cells ranged from 7 (which appeared to be an outlier) to 95%, with a mean of 61% (excluding the aberrant low case). It is important to note that many of the samples contained significant numbers of immunohistochemistry-proven histiocytes,[17] indicating that total BDL cell count

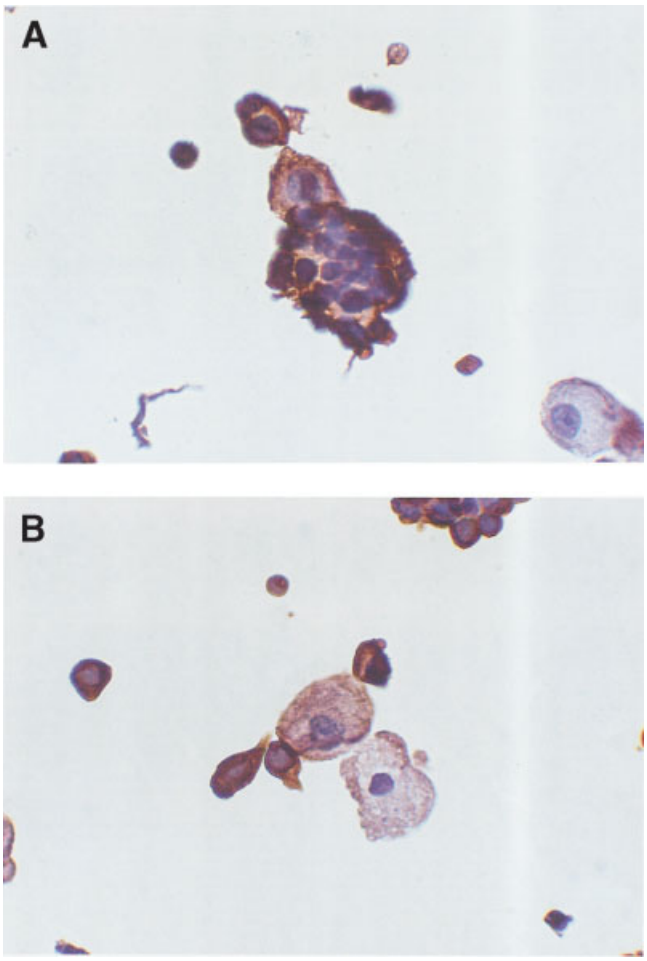

Fig. C-1. A and **B:** AE1/AE3 staining of breast duct epithelial cells, which are present singly and in clusters. Nonstaining histiocytes are present (DAB and hematoxylin, ×600).

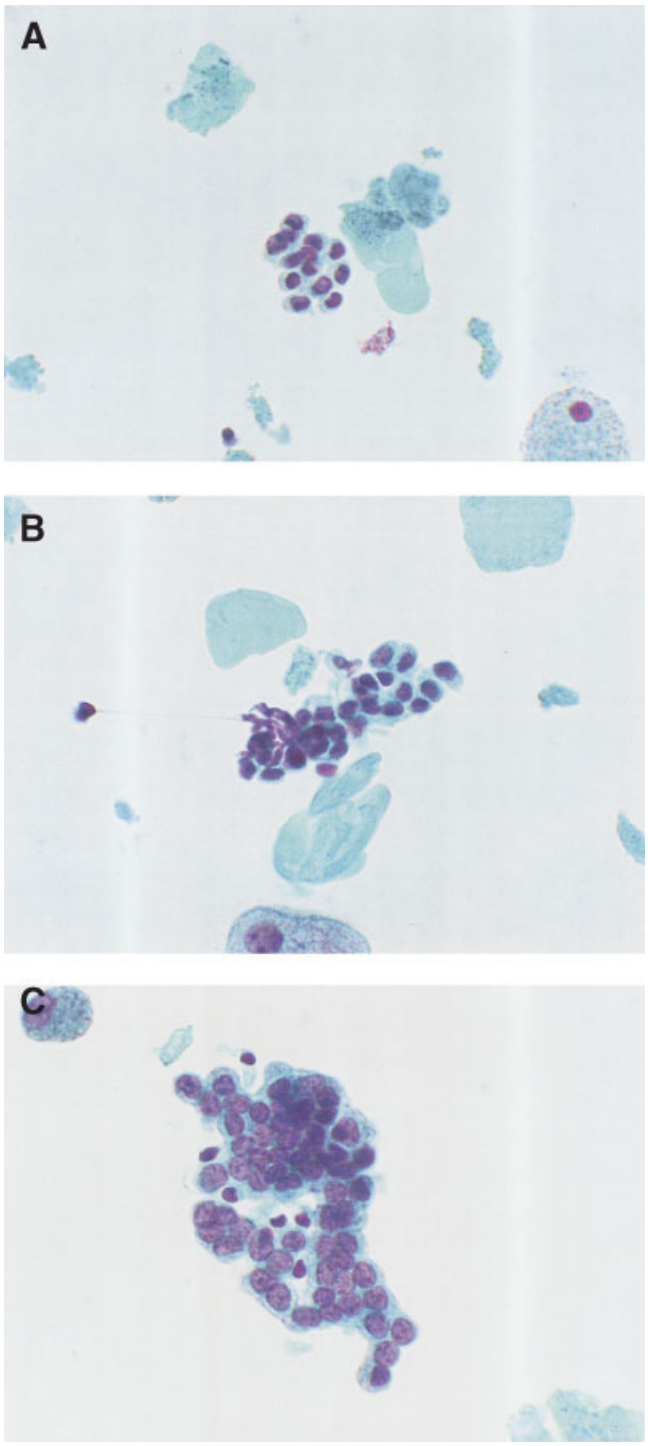

Fig. C-2. A, **B**, and **C:** Clusters of breast duct epithelial cells comprising approximately 10+, 20+, and 50+ cells (Pap, ×600).

is not an accurate indicator of the actual number of breast duct epithelial cells in a given sample.

We have devised a simple formula that can be used in the vast majority of cases for the accurate assessment of BDL cellularity. This formula could be used to standardize enumeration of BDL cell counts for interinstitutional and interoperator comparisons of this new technique. Our preliminary results suggest that BDL is unlikely to be a source of large numbers of cells for either diagnostic or research purposes. Furthermore, the population of cells that is obtained is heterogeneous, with varying degrees of contamination by histiocytes. These latter cells are less likely to be informative for diagnostic cytology or biomarker research. Investigators who plan to employ BDL samples for research purposes must take this cellular heterogeneity into account to insure meaningful results.

Questions regarding the ultimate utility of BDL will hopefully be answered by the results of a large, multicenter BDL trial (the "Serial Evaluation of Ductal Epithelium" trial).[13] This trial has been designed to investigate cytologic features and novel markers of BDL cells, as well as to evaluate the following issues[13]:

- optimal frequency for the performance of BDL in high-risk women

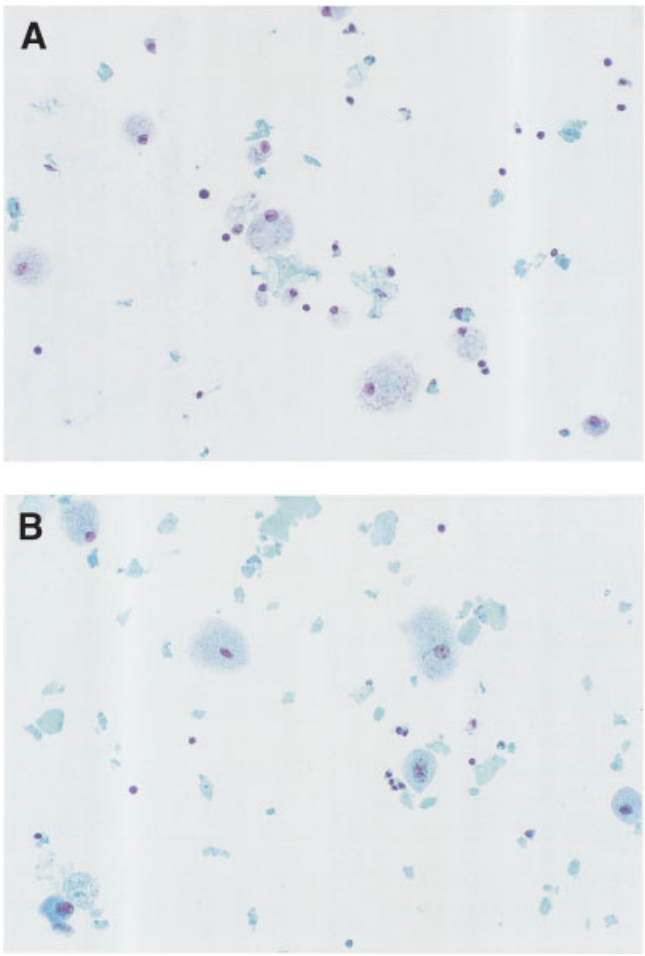

Fig. C-3. A and **B:** Approximate cell counts (A) 18 epithelial cells, 12 histiocytes; (B) 12 epithelial cells, 6 histiocytes (Pap, ×20).

- negative predictive value of duct lavage cytology
- significance of NAF yielding versus nonyielding ducts
- ability of cytology to gauge the risk of a high-risk woman
- role of molecular markers in addition to or in lieu of cytologic evaluation.

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